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blood samples for the diagnosis of AIDS-related cerebral toxoplasmosis: a case-control study

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ABSTRACT

Background: Cerebral toxoplasmosis (CT) continues to cause significant morbidity and mortality in human immunodeficiency virus (HIV)-infected patients in Brazil. In clinical practice, the initial diagnosis is usually presumptive and alternative diagnosis tools are necessary. Our objective was to evaluate whether the detection of high titers of IgG anti-*Toxoplasma gondii* and *T. gondii* DNA in blood samples are associated with the diagnosis of CT. **Methods:** In this case-control study we included 192 patients with HIV-1 infection: 64 patients with presumptive CT (cases) and 128 patients with other diseases (controls). Blood samples to perform indirect immunofluorescense reaction (IFI) to detect anti-*T. gondii* IgG antibodies and polymerase chain reaction (PCR) were collected before or within the first three days of anti-*Toxoplasma* therapy. Two multivariate logistic regression models were performed: one including the variable qualitative serology and another including quantitative serology. **Results:** In the first model, positive IgG anti-*T. gondii* (OR 4.7, 95% CI 1.2-18.3; p = 0.027) and a positive *T. gondii* PCR result (OR 132, 95% CI 35-505; p < 0.001) were associated with the diagnosis. In the second model, IgG anti-*T. gondii* titres ≥ 1:1024 (OR 7.6, 95% CI 2.3-25.1; p = 0.001) and a positive *T. gondii* PCR result (OR 147, 95% CI 35-613; p < 0.001) were associated with the diagnosis. **Conclusions:** Quantitative serology and molecular diagnosis in peripheral blood samples were independently associated with the diagnosis of CT in HIV-infected patients. These diagnostic tools can contribute to a timely diagnosis of CT in settings where *Toxoplasma* infection is common in the general population.

Keywords: toxoplasmosis; cerebral toxoplasmosis; polymerase chain reaction; diagnosis; acquired immunodeficiency syndrome; Brazil.

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) has resulted in a dramatic reduction of the incidence of acquired immunodeficiency syndrome (AIDS)-related cerebral toxoplasmosis (CT) in developed countries.[1] Similarly, an important decrease in case reports have been observed in São Paulo in the HAART era. Nevertheless, CT continues to cause important mortality and morbidity in our settings.[2] In clinical practice, presumptive diagnosis of CT is based on clinical and radiological features associated to successful response within 14 days of specific treatment.[3,4] On the other hand, definitive diagnosis using biological tools (intraperitoneal inoculation to laboratory animals or inoculation to cell cultures *in vitro*) showed low sensitivity[5] and brain biopsy has complications and elevated costs.[6] Thus, alternative approaches for a timely diagnosis are necessary. In recent years, molecular diagnosis of CT in cerebrospinal fluid (CSF) and peripheral blood samples had significant improvement,[5] but has not produced conclusive results.[4] Here, we evaluated whether detection of high titers of IgG anti-*Toxoplasma gondii* and *T. gondii* DNA in blood samples could be used to diagnose CT.

PATIENTS AND METHODS

All patients were admitted to *Instituto de Infectologia Emílio Ribas,* a referral tertiary hospital in São Paulo, Brazil. This case-control study included 192 patients with HIV-1 infection documented by antibody enzyme-linked immunosorbent assay (ELISA) and Western Blot tests: 64 patients with presumptive cerebral toxoplasmosis (cases) and 128 patients with other

diseases (controls). Cases were defined as follows: I) recent onset of a consistent focal neurological abnormality with intracranial disease or reduced level of consciousness; II) a lesion having a mass effect evidenced by brain imaging (on computed tomography or magnetic resonance) or a lesion whose radiographic appearance was enhanced by injection of contrast medium, and III) successful response to the specific treatment. The control group consisted of 128 patients with other diseases. Sixty-four presented with neurological diseases: 25 with cryptococcal meningoencephalitis, 7 with progressive multifocal leukoencephalopathy, 14 with central nervous system (CNS) tuberculosis, 12 with HIV-associated neurocognitive disorders, and six with syphilitic meningitis. The other 64 patients presented with non-neurological diseases: 18 with pulmonary tuberculosis, 12 with bacterial pneumonia, seven with oral candidiasis, five with diffuse lymphoma, and 22 with *Pneumocystis jiroveci* pneumonia. Blood samples to perform indirect immunofluorescense reaction (IFI) to detect anti-*T. gondii* IgG antibodies and polymerase chain reaction (PCR) were collected before or within the first three days of anti-*Toxoplasma* therapy. IgG *T. gondii* titers ≥ 1:16 and ≥ 1:1024 were considered positive and high, respectively. The samples were analyzed by conventional PCR targeting a 115-base-pair sequence in a specific repetitive region of the *B1* gene of *T. gondii*. PCR and IFI methodologies were reported elsewhere.[7]

For statistical analysis, the outcome variable in the present study was the diagnosis of CT. Univariate logistic regression analysis was performed to identify the independent variables that were associated with the diagnosis of CT. The list of potential variables included age, gender, prior CT, lymphocyte T-CD4+ cell count, use of CT prophylaxis at admission, positive IgG anti-*T. gondii* IFI, high titers of IgG anti-*T. gondii* IFI, and a positive PCR result for *T. gondii* DNA in blood samples. Only those variables with a p-value ≤ 0.2 were included in the multivariate analysis. Variables with a p-value ≤ 0.05 remained in the final multivariate logistic regression model. Correlations between qualitative serology (IFI ≥ 1:16) and quantitative serology (≥ 1:1024), and between quantitative serology (≥ 1:1024) and a positive *T. gondii* PCR were calculated with the nonparametric correlation Spearman's test. Statistical analysis was performed using SPSS software 10.0 (SPSS Inc. Product Registration, Chicago, USA, 1999). This study was approved by the institutional review board of *Instituto de Infectologia Emílio Ribas*.

RESULTS

The serological prevalence of *T. gondii* infection in the patients included in this study was 68% [58/64 (91%) of cases and 72/128 (56%) of controls (p < 0.001)]. When only patients with a positive result of IgG anti-*T. gondii* IFI we compared, cases (52/58, 90%) presented more frequently high IgG titers than controls (39/72, 54%) (p < 0.001).

According to the primary criteria in univariate analysis (Table 1), the following variables were associated with the

Table 1. Univariate analysis for the identification of independent variables associated with the diagnosis of cerebral toxoplasmosis

Variable	Cases (n = 64)	Controls (n = 128)	OR (95% CI)	p
Age (years)				
Mean ± SD	35.9 ± 9.5	35.8 ± 7.7	1.0 (0.9-1.0)	0.902
Gender				
Male	37	78	0.9 (0.5-1.6)	0.677
Female	27	50		
Previous cerebral toxoplasmosis				
Yes	11	16	1.5 (0.6-3.4)	0.380
No	53	112		
CD4 T-cell count (cells/µL)				
Mean ± SD	71.4 ± 72.9	61.5 ± 0.6	1.0 (0.9-1.0)	0.284
Use of prophylaxis at admission				
Yes	3	23	0.2 (0.1-0.8)	0.019
No	61	105		
IgG anti-*T. gondii*				
≥ 1:16	58	72	7.5 (3.0-18.7)	< 0.001
< 1:16	6	56		
IgG anti-*T. gondii*				
≥ 1:1024	52	41	9.2 (4.4-19.1)	< 0.001
< 1:1024	12	87		
T. gondii PCR in blood				
Positive	51	3	163 (45-597)	< 0.001
Negative	13	125		

diagnosis of CT: no use of CT prophylaxis at admission, IgG anti-*T. gondii* IFI ≥ 1:16 or IgG anti-*T. gondii* IFI ≥ 1:1024, and a positive *T. gondii* PCR result. Nonparametric correlations between qualitative and quantitative serology were high ($p < 0.001$). For this reason, were designed two multivariate logistic regression models: one including the variable qualitative serology and another including quantitative serology. In the first case, only positive *T. gondii* IFI [OR 4.7, 95% CI 1.2-18.3; $p = 0.027$] and a positive *T. gondii* PCR result [OR 132, 95% CI 35-505; $p < 0.001$] remained in the final model. In the latter model, only IgG *T. gondii* IFI ≥ 1:1024 [OR 7.6, 95% CI 2.3-25.1; $p = 0.001$] and a positive *T. gondii* PCR result [OR 147, 95% CI 35-613; $p < 0.001$] remained in the final model. In addition, nonparametric correlations between qualitative serology and a positive *T. gondii* PCR result, and quantitative serology and a positive *T. gondii* PCR result were high ($p < 0.001$).

DISCUSSION

The main finding of our study, performed in a setting with high prevalence of *Toxoplasma* infection in the general population, was the significant association between high IgG anti-*T. gondii* titers and PCR detection of *T. gondii* DNA in peripheral blood samples and the diagnosis of CT.

Cerebral toxoplasmosis is the most frequent CNS opportunistic infection in HIV-infected patients in Brazil.[8,9] In a previous report, we observed that CT accounts for 10% of the admissions at *Instituto de Infectologia Emílio Ribas*, usually occurring as AIDS-defining disease.[2] In addition, similarly to other reports,[10] CT continue to appear in Brazil among patients with late HIV diagnosis, with poor compliance with HAART, or with treatment failure.

In the present study, we identified that non-compliance with CT prophylaxis at admission was predictive of CT in univariate analysis. The benefit of prophylaxis is well described in the literature; therefore, it is possible that this variable did not remain significant when included in the multivariate analysis due to the low proportion of controls using antibiotic prophylaxis at admission. Classically, positive IgG *T. gondii* serology has been considered in the diagnosis of CT.[11] In a large study, Raffi et al.[12] reported a trend towards higher median IgG anti-*T. gondii* titers in patients with CT ($p = 0.08$). However, only qualitative *T. gondii* serology, but not the quantitative one, was associated to the diagnosis of CT. In accordance with studies that showed the predictive value of high IgG anti-*T. gondii* titers,[13,14] our results showed an independent association between high titers of IgG anti-*T. gondii* and the diagnosis of CT. This association could reflect the direct effect of qualitative serology, as it was demonstrated with the high correlation between qualitative and quantitative serologies. Nevertheless, when we analyzed only the patients with a positive serology, cases had a frequency of elevated IgG titers higher than controls, suggesting the additional value of quantitative serology.

Available studies of PCR using peripheral blood in HIV-related CT and usually performed with conventional PCR protocols and small number of subjects, reported sensitivities ranging from 13% to 88% with specificity ≥ 95%.[5,15] Recently, we reported our experience with conventional PCR assay using peripheral blood samples in patients with AIDS-related CT, showing a sensitivity of 80% and a specificity of 98%.[7] These very divergent results can be due, at least in part, to several reasons: choice of target DNA and primers, PCR techniques, conditions and time of sample storage, and collection of blood samples before or in the first days after introduction of specific treatment.[5,15,16] It was reported that genotypes of *T. gondii* strains isolated from patients with ocular toxoplasmosis in Brazil were highly divergent when compared to the previously described clonal lineages from North America and Europe, suggesting more frequent sexual recombinants resulting in mixed genotypes.[17] Similarly, our group showed the high rate of genetic exchange in *T. gondii* strains isolated from HIV-infected patients with CT.[18] Future studies should evaluate the relation between genetic diversity, parasitic burden, molecular diagnosis and clinical outcomes in AIDS patients with CT.

Introduction of real-time PCR and automated DNA extraction could avoid some limitations of conventional PCR and perform a more accurate and timely diagnosis.[15] A recent report of our group found high sensitivity of real-time quantitative PCR, in CT diagnosis, using two different primer sets (*B1*Tg, which amplified a sequence from the *B1* gene and RETg, which amplified a PCR product of the 529 base pairs sequence).[19]

Reinforcing the importance of molecular diagnosis using peripheral blood samples in immunedeficient patients, a study of patients with allogenic stem cell transplantation suggested that quantitative PCR assay may guide preemptive therapy and avoid death due to toxoplasmosis in about 80% of patients who develop infection.[20]

International recommendations of diagnosis and management of expansive brain lesions in HIV-infected patients consider primary CNS lymphoma (PCNSL) as the second most frequent opportunistic disease in developed countries.[4,21] However, PCNSL is uncommon in most developing countries, where focal forms of CNS tuberculosis (i.e. tuberculomas) are the main differential diagnosis of CT.[22,23] Furthermore, the clinical and radiological differentiation between these opportunistic infections poses important challenges in resource-limited settings, where both infections are highly prevalent in the general population and alternative and timely diagnosis approach are necessary.[24]

In accordance with a previous study carried out in a middle-income country, our findings demonstrate that PCR is a relatively simple and rapid procedure that can be performed in developing countries with reasonable laboratory infrastructure.[25] The results of the present study highlight

the importance of considering quantitative serology and molecular diagnosis in peripheral blood samples in the initial management of HIV-infected presenting with expansive brain lesions. In this line, a recent algorithm incorporated these tools.[26]

In conclusion, our results suggest that high IgG anti-*Toxoplasma* titers and a positive PCR result for of *T. gondii* DNA in blood samples were significantly associated with CT in AIDS patients. These diagnostic tools must be interpreted in association with clinical and radiological information, and can contribute to a timely diagnosis, especially in Brazil and in other low and middle-income countries with high prevalence rates of *Toxoplasma* infection in the general population.

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